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# Predator-Induced Plasticity in Spotted Salamanders (Ambystoma maculatum)

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# Abstract

The ability to respond plastically to the environment has allowed amphibians to evolve adaptive responses to spatial and temporal variation in predation threat. However, animals exposed to predators may also show costs of plasticity or tradeoffs. This study examines predator-induced plasticity in larval development, behavior, and metamorphosis in the spotted salamander, *Ambystoma maculatum*. Salamanders were raised in two treatments: with predator cues (a fish predator, genus *Lepomis*, on the other side of a divided tank), or without predator cues. During the larval stage the predator treatment group experienced higher mortality rates than the no-predator treatment group. Behavioral trials revealed that predator treatment animals ate less than those not exposed, and that this feeding response was immediately inducible and had lasting effects. Animals in the predator treatment group had smaller tail areas during the mid-larval period. Feeding and body size effects may have contributed to increased mortality in the predator-treatment animals. The timing of metamorphic onset was not affected by the presence of predators, but predator-treatment salamanders had shorter snout/vent lengths at metamorphosis. The duration of metamorphosis showed a potentially adaptive plastic response to the presence of predator cues: metamorphosis was longest in the no-predator treatment group, reduced in the predator treatment group, and even further reduced for animals exposed to predator cues only during metamorphosis. Overall, we found a mix of potentially adaptive and costly plastic responses in spotted salamanders.

# Introduction

Plasticity is the ability of an organism to alter its phenotype, development, or behavior in response to the environment. Amphibians are known to be extremely sensitive to a variety of environmental cues (Rose 2005). The capacity to plastically respond to conditions around them allows amphibians to utilize habitats that are often extremely heterogeneous or variable spatially or temporally. Many types of plastic responses have been well studied in amphibians, but these studies have largely focused on anurans. In addition, the process of metamorphosis as a potentially plastic life stage has also been neglected (Downie et al. 2004).

Amphibians have been shown to exhibit plasticity in response to environmental factors such as crowding, predators, food resource levels, pond drying, and temperature, and to respond during hatching, the larval period, or metamorphosis. Predation risk is an important component of the environment that can induce behavioral, developmental, or morphological plastic responses, but these defenses may come with costs or tradeoffs. Costs of predator-induced plasticity may include reduced larval survival, lowered growth rate, (McCollum and Van Buskirk 1996, Van Buskirk 2000), or increased susceptibility to predators at a later life stage (Benard and Fordyce 2003).

Many anuran species show phenotypic plasticity in the presence of predators. A distinctive morphology is often induced in the presence of predators; relatively shorter, deeper tail fins are often brightly colored with dark spots (Van Buskirk 2002, McCollum and Van Buskirk 1996, Schoeppner and Relyea 2008a, 2008b, Van Buskirk et al. 2004). This type of induced phenotype may be an adaptive response because it draws predator strikes to the tail and spares the more vulnerable body core (Van Buskirk et al. 2003, Van Buskirk et al. 2004). Alternatively, studies in the gray tree frog (*Hyla versicolor*), agile frog (*Rana dalmatina*) and

southern leopard frog (*Rana sphenocephala*), showed that longer tails with deep fins are induced by the presence of predator cues, and that this morphology increases escape performance (Van Buskirk and McCollum 1999, 2000, Teplitsky et al. 2005, Johnson et al. 2008). In these cases, vulnerability to predation during the larval stage has driven the evolution of phenotypic plasticity.

Behavioral plasticity has also been well studied in many larval amphibians. Wood frogs (*Rana sylvatica*) and American toads (*Bufo americanus*) have been shown to increase hiding behavior and decrease activity rates in the presence of predators (Schoeppner and Relyea 2008a, 2008b, Skelly and Werner 1990, Smith et al. 2008, Sih et al. 1988). A study by Orizaola and Braña (2003) found only hiding activity to be affected. In general, in the presence of predation threat, reduced activity and foraging, accompanied by more refuge use or hiding, are potentially adaptive because they reduce susceptibility to attack by predators. However, this plasticity engenders costs in some species – reduced feeding causes reduced size or growth rates, which could delay the onset of metamorphosis (Skelly and Werner 1990).

Amphibians have also been shown to exhibit developmental plasticity at ontogenetic niche shifts (life history switch points). Hatching plasticity, the ability of embryos to hatch out early in response to an environmental cue, especially from predators, is a strategy to avoid immediate risk and has been well-studied in amphibians. After a certain stage, after which embryos are viable outside the egg in the water, embryos will hatch out in response to egg predators (e.g., snakes, wasps, amphibians; Warkentin 1995, 2000, Vonesh 2005, reviewed in Wells 2007) or fungal infections (Warkentin et al. 2001). Hatching may be delayed in the presence of a larval predator (Sih and Moore 1993), but immediate threats to embryos take precedence over potential future threats to larvae (Brown-Wilusz and Landberg, unpublished

2008). Costs of early hatching are manifested in embryos that hatch at smaller sizes and earlier stages of development, and are more vulnerable to larval predators.

The larval developmental rate and thus the onset of metamorphosis are also capable of responding to predators. Development to metamorphosis may be accelerated in ephemeral environments, when pond drying poses a threat, but this adaptive response comes at the cost of decreased size at metamorphosis (Newman 1989, 1992). During the larval period, theory predicts that predation pressure will cause amphibians to accelerate development and initiate metamorphosis earlier and at smaller body size (Werner 1986). Observed responses have not always matched these predictions. Red-eyed tree frogs, *Agalychnis callidryas*, accelerate the rate of larval development in the presence of predaceous giant water bugs (*Belostoma* spp.; Vonesh and Warkentin 2006), while Relyea (2007) reports that most caged-predator studies that examined the effects of predator cues independent of other predator effects, found that prey species did not initiate metamorphosis earlier or at a different size.

Alternatively, the process of metamorphosis itself may manifest predator-induced plasticity. Metamorphs are considered more vulnerable to predation by aquatic predators than larvae, since in this transitional stage their locomotor performance is diminished (Arnold and Wassersug 1977, Rose 2005, Walsh et al 2008a). Therefore theory predicts that selection should have minimized the total duration of metamorphosis. However, the ability to plastically alter the duration of metamorphosis could be an adaptive response if predation threat is not constant during this transitional process, as is the case in many amphibian habitats (Wassersug and Sperry 1977). In the African clawed toad, *Xenopus laevis*, the duration of metamorphosis was reduced in response to predation (Walsh et al. 2008b). However, the potential for plasticity of metamorphosis has not been studied widely (Downie et al. 2004).

In this study, we raised spotted salamanders, *Ambystoma maculatum*, either with or without cues from a sunfish (*Lepomis* spp.) predator. We measured developmental stage, mortality, body size, feeding and hiding behavior, timing of and size at metamorphosis, and the duration of metamorphosis for plasticity. The study aimed to identify evidence of adaptive plastic responses, or costs and tradeoffs associated with exposure to predators. Adaptive responses would be expected to reduce the overall time spent in the presence of the predator, such as hiding, shortening the larval period and initiating metamorphosis early, or reducing the duration of metamorphosis. Potential costs would be observed in reduced survival, development, or feeding, lower body size, or poor condition.

## Materials and Methods

*Experimental Design:*

This study used a split-clutch, randomized block design. Six egg masses (clutches) of spotted salamander, *Ambystoma maculatum*, eggs were collected from the Fenton River, Storrs, CT (April 24, 2008), and distributed into forty-eight 38 liter (10 gallon) aquaria. Each of the six egg masses were divided into eight approximately equal sections that contained 19 ± 0.3 (mean ± SE) eggs, with salamander embryos ranging in stage from 20-35 (Harrison, 1969). Each of these sections was assigned randomly to one of two treatments, no-predator (hereafter NP) or predator (hereafter P, containing sunfish: *Lepomis macrochirus* or *L. cyanellus*). There were four replicates per treatment: two replicates within each of two spatial blocks, with tanks randomly assigned to a treatment. Fish were supplied by the Connecticut Department of Environmental Protection and the Kentucky Department of Fish and Wildlife.

Due to disproportionate mortality experienced by the P treatment group, salamanders from NP tanks were moved to P tanks within clutch and block three times, on June 23, July 24, and July 28, to maintain more even salamander densities across tanks. This redistribution would tend to bias this study against detecting treatment effects.

*Animal Husbandry:*

All animals were maintained in an Aquatic Animals Facility (Room 106) in the Torrey Life Sciences Building of the University of Connecticut, Storrs, CT. Air temperature was kept at 12°C until June, and was then increased two degrees per week throughout the summer. Throughout the experiment, the animals experienced a 12 hour light: 12 hour dark cycle. All tanks were divided into two equal halves by a fiberglass screen with 2 mm pores (Figure 1A), which allowed visual and chemical predator cues to reach the salamanders, but prevented fish

from eating the salamanders. The P treatment tanks had a sunfish in the rear half of the tank, while the NP treatment tanks did not. All aquaria were filled with distilled water mixed with RO Right brand salt (1 tbsp/5 gallons) to match the salinity of normal freshwater conditions. Several terra cotta pieces were placed on the bottom of the tank, to provide a substrate in which the salamander larvae could hide. Air was constantly supplied to all tanks from a bubbler connected to a pump. Water, with any dirt or debris, was changed once per week or as necessary. Tanks were checked daily, any dead fish were promptly removed, and the tank was bleached and washed before addition of new water and reinstatement of the salamanders. Live invertebrates (primarily chironomid larvae and oligochaetes) were collected from the Quinnebaug Valley Trout Hatchery weekly and supplied to the salamanders every two to three days. Fish were fed a mixture of aquatic invertebrates, *Ambystoma maculatum* larvae, *Rana* tadpoles, and earthworms.

When a salamander initiated metamorphosis, it was removed from its home tank and placed individually in an acrylic aquarium ("box") (10x10x6 cm) containing water from the experimental tanks and a concave shard of terra cotta, which salamanders could utilize as a hiding place and to climb out of the water. Animals from the NP treatment were randomly assigned to a box containing either no-predator water or predator water. This created a new "box treatment:" no predator-predator (hereafter NP-P). All P treatment salamanders continued to be housed in predator water during metamorphosis. Water was changed every third day. NP treatment boxes received a mixture of water from each of the NP tanks, while NP-P and P treatment boxes received a mixture of water from each of the P tanks.

At the end of the experiment (December 22, 2008, Day 203), all salamanders were overdosed with buffered 2 g/L tricaine methanosulfonate (MS-222), and preserved in formalin.

*Staging:*

Animals were staged according to a modified staging table based on Harrison (1969). Harrison's stages stop at stage 46, in which animals have two toes and one toe bud on the front limbs, and one toe bud on the hind limbs. A toe bud is longer than it is wide, and then elongates into a toe. Previous research (Brown-Wilusz and Landberg, unpublished, 2008) extended this table to include stages 47 to 55, using the additional development of toe buds to toes (four total front limb toes and five total hind limb toes) (Table 1). This staging table was extended to include an additional 6 stages which encompass further larval development and metamorphosis. These stages are defined based on visible external changes in skin pigmentation, gill resorption, and tail fin resorption, and are summarized in Table 1.

*Development:*

The number of animals hatched was recorded weekly until June 2, 2008, when all animals had hatched (designated day 0 of the experiment). All salamanders were counted and staged approximately every two weeks, a total of ten times over 90 days, and the mean stage was calculated for each tank. From these counts, the death rate was calculated as proportion dead per day ($[n_{\text{previous date}} - n_{\text{current}}]$/duration).

As individuals neared metamorphosis, tanks were checked daily and any individuals at stage 56 were transferred to individual boxes. All metamorphs were staged daily according to the extended metamorphic staging table.

*Behavioral Trials:*

*Home water* (7/9/08, Day 37): One haphazardly chosen salamander from each tank was placed into an acrylic "box" (10x10x6 cm), containing water and a piece of terra cotta from that animal's home tank, and exactly five bloodworms (n=38 boxes) (Figure 1B). Each animal was

staged as it was placed into its corresponding box. The trial lasted 90 minutes for each salamander, and the animals were not disturbed for the duration of the trial. At the end of the trial, whether or not the salamander was hiding (at least 50% of body under the terra cotta piece) and the number of worms that had been eaten were recorded for each animal. After completion of the trial, all animals were returned to their home tanks.

*Opposite water* (7/22/08, Day 50): The behavior trial was repeated as above, but all animals were placed into boxes containing water and a piece of clay from randomly assigned tanks of the opposite treatment (n=35 boxes). Thus, NP treatment salamanders experienced novel chemical predator cues, and P treatment animals experienced a lack of predator cues for the duration of the experiment. Additionally, all aquaria were covered and left over night. The next morning, at the end of 1,350 minutes (22.5 hours) since the start of the behavior trial, hiding and the number of worms eaten was recorded once again.

*Home water vs. foreign water* (7/29/08, Day 57): In order to rule out the possibility that any changes in behavior observed during the trials was due to receiving water that was simply different than the home water and not of a different treatment in particular, we conducted another set of behavioral trials. Two salamanders were haphazardly chosen from each tank containing at least two salamanders. One salamander from each tank was placed into a box containing water and clay from the home tank while the other salamander was placed into a box containing water and clay from a foreign tank, that is, a different, randomly chosen tank from the same treatment (n=56 boxes). The trial proceeded as described previously: hiding and the number of worms eaten was recorded for each box at the end of 90 minutes.

*Foreign water vs. opposite water* (7/31/08, Day 59): This trial was repeated as described directly above for the "home water vs. foreign water" trial, however one salamander from each

tank was placed into a box containing foreign water (same treatment), while the other salamander was placed into a box containing water from a randomly assigned tank of the opposite treatment (n=56 boxes). The trial proceeded as previously described for 90 minutes, and hiding and the number of worms eaten was recorded.

*Morphometrics:*

Body size measurements were taken from high resolution digital lateral-view photographs taken in the small acrylic boxes (10x10x6 cm) that had a 2 cm scale bar for calibration. These photographs were analyzed using Image J software for several traits: total length, snout-vent length, tail length, maximum tail height, and tail area (i.e. Azizi and Landberg, 2002). These photographs were taken at the time of the behavioral trials (7/10/08, Day 38) during the mid larval period. In addition, all new metamorphs (stage 56) were photographed before they were placed into their individual boxes.

At the time of the behavioral trials, on 7/10/08 every salamander was weighed. Individuals were patted dry with a piece of paper towel, and then weighed on a slip of paper towel on a digital balance. The paper towel was then weighed alone and the difference was recorded as the weight of the animal. Each animal was then returned to its home tank.

*Statistical Analysis:*

Statistical analyses were conducted using JMP 5.0 software. Tank mean values were used in all analyses (prior to metamorphosis), since tanks were our unit of replication, not individuals. A linear model analysis of variance (ANOVA) was used in all tests, and significant differences were determined using Tukey's post-hoc test of tank means which were conducted for all analyses, with clutch, block, and treatment as fixed factors. Day of the experiment, stage,

and body size were covariates when appropriate. Individuals were the units of replication used in the analysis of the duration of metamorphosis.

# Results

*Larval Development and Survival*

P and NP treatment groups did not differ in their rate of development during the larval stages, with day as a covariate (ANCOVA, p=0.68, Tukey post-hoc test, Table 2, Figure 2). Clutches began at different stages.

Average death rate per day was significantly affected by treatment, day, and the interaction of treatment and day (Table 2). Death rate declined over time in both treatments; however, animals in the P treatment had a significantly higher death rate than those in the NP treatment (ANCOVA, $p<0.0001$, Tukey post-hoc test, Figure 3).

*Behavioral Trials*

*Home water* (7/9/08, Day 37): The NP treatment group ate significantly more worms in home water than the P treatment group (ANCOVA, p=0.0003, Tukey post-hoc test, Table 3). NP treatment animals tended to hide more, but the difference was not significant (p=0.09; Table 4; Figure 4A).

*Opposite water* (7/22/08, Day 50): Salamander larvae exposed to water of the opposite treatment for either 1.5 or 22.5 hours did not show differences in the number of worms eaten between treatments. The NP treatment group reduced its feeding rate to levels similar to the P treatment group. The number of worms eaten did not differ between treatments for either time span (1.5 hrs: p=0.63; Table 2; 22.5 hrs: p=0.63; Table 3). Hiding was also not affected by treatment (nominal logistic fit test; 1.5 hrs: p=0.61; 22.5 hrs: p=0.14; Table 4).

*Home water vs. foreign water* (7/29/08, Day 57): The number of worms eaten did not differ between water type (home water or foreign water from a different tank of the same

treatment) for either treatment (ANCOVA, Table 3, Figure 4B). Hiding behavior did not differ between water types within treatment (nominal logistic fit test; p=0.28; Table 4).

*Foreign water vs. opposite water* (7/31/08, Day 59): The treatment*water type interaction was highly significant for number of worms eaten indicating that animals in the two treatments responded differently to foreign vs. opposite water type ($p<0.0001$, Table 3). For the NP treatment group, the number of worms eaten was significantly higher in foreign (NP) water than in opposite (P) water (Figure 4C). Within the P treatment group, there was no difference in feeding between the two water types (Figure 4C), nor was the number of worms eaten by the P treatment animals different from the number eaten by the NP treatment group in P water. There was no difference in hiding between water type within treatment (nominal logistic fit test; p=0.30; Table 4).

*Morphology and body weight*:

At the time of the behavioral trials, on 7/10/08, the P and NP treatment groups did not differ in mass (ANCOVA, p=0.48, Table 5). The NP treatment group had larger relative tank mean tail areas (corrected for total length) than the P treatment group (ANCOVA, p=0.0024, Tukey post-hoc test, Table 5, Figure 4D). No other size measurements showed significant effects of treatment.

*Onset of Metamorphosis*

The tank mean age at the onset of metamorphosis (Stage 56) was not different for NP and P treatment salamanders (ANCOVA, p. = 0.41, Table 6, Figure 5A). The snout/vent length at the time of onset of metamorphosis was affected by treatment (ANCOVA, p=0.017, Table 6). The NP treatment group was larger at metamorphic onset (Tukey post-hoc test; Figure 5B).

Snout-vent length and tail length had significant effects on the age at onset of metamorphosis (ANCOVA; $p=0.0008$, $p<0.0001$, respectively; Table 6), but they act in opposite directions. Tail length is inversely related to mean age at metamorphosis, across treatments (Figure 5C), while snout-vent length is directly related to the age of metamorphic onset (Figure 5D).

*Duration of Metamorphosis*:

The duration of metamorphosis was examined in terms of box treatment (NP, NP-P, or P). The duration of metamorphosis was significantly affected by the box treatment nested within tank treatment (ANCOVA, $p=0.004$, Tukey post-hoc test; Table 6, Figure 6). All three box treatments had statistically different mean durations of metamorphosis. The duration of metamorphosis was highest in the NP treatment, reduced in the P treatment, and further reduced in the NP-P treatment.

## Discussion

Exposure to fish predator cues throughout development significantly affects larval survival, feeding behavior, relative tail area, size at metamorphosis, and the duration of metamorphosis in spotted salamanders (*Ambystoma maculatum*).

Survival of salamanders exposed to fish predator cues was dramatically lower than for those not exposed; the death rate was approximately twice as high in the predator (P) treatment group as in the no-predator (NP) treatment group (Figure 3). Since fish were isolated from the experimental salamanders, this mortality was not a result of predation. Instead, it appears to be an effect of perception of chemical and/or visual cues.

Gray tree frog (*Hyla chrysoscelis*) tadpoles exposed to caged predators also had lower survival (McCollum and Van Buskirk 1996). Contradictory to these results, a study of the response of *A. maculatum* actually exposed to *Lepomis macrochirus* predators, found that the presence of fish did not affect larval survival (Figiel and Semlitsch 1990). In that study, there was no difference in mortality between populations raised in the presence or absence of predators, but the highest mortality occurred in salamanders from source ponds that contained sunfish, suggesting that survival may be related to the historical predator environment. In our study, all salamanders came from the same source, which may have allowed effects of predator presence for the duration of larval development to emerge. Figiel and Semlitsch also found that resource (zooplankton) density was lower in experimental pools with fish. It is possible that sunfish preferentially fed on zooplankton, especially since salamander larvae decreased their activity and hid.

It is possible that in our study the high level of chemical cues led to an unnaturally high stress response (scared to death!), although not all salamanders in the P treatment died. Other

possible contributors include fish death affecting water quality, or perhaps chemicals released by stressed fish.

For those animals surviving in the P treatment, the rate of development during the larval period was not affected by the presence of predator visual and chemical cues (Figure 2). This is in agreement with the previous study by McCollum and Van Buskirk (1996), who found that gray tree frogs in the presence of a caged odonate predator also develop at the same rate as those not exposed. This suggests that the rate of larval development is not accelerated by the perception of larval predators. Reducing the amount of time spent in a risky larval environment (such as one containing predators) would be adaptive if there was no cost associated with doing so. Since we know that some environments do cause an acceleration of larval development (Newman 1989, Vonesh and Warkentin 2006), these results suggest that *A. maculatum* are either unable to increase accelerate development due to physiological constraints or because a cost of doing so prohibits it.

Amphibians have been shown to frequently exhibit behavioral plasticity in response to predation threat. Our expectation, based on previous studies, was that salamanders would decrease their active feeding behavior and increase their hiding behavior, or refuge use to avoid detection by predators (Schoeppner and Relyea 2008). Hiding behavior was never affected by the presence or absence of chemical predator cues (Figure 4A), in contrast to many previous studies, in which hiding increased in the presence of predators or predator cues (Sih, Kats, and Moore 1992, Sih, Petranka, and Kats 1988, Orizaola and Braña 2003). A study by Walls (1995) compared *A. talpoideum* to *A. maculatum* in the presence or absence of a mutual predator, and found that refuge use did not increase in the presence of a larval predator (*Ambystoma opacum*) for *A. maculatum*. *A. maculatum* is a superior forager, so a possible explanation is that in the

presence of predation and competition, they took advantage of their competitive advantage in feeding success despite the predation risk. In addition, our trials only used chemical predator cues. It is possible that the addition of visual cues might have elicited a hiding response.

In their home tank water, that is, the water that the larvae were raised in, larvae exposed to predator cues ate significantly less during behavioral trials. This matched our predictions, and was in agreement with previous studies that also found a decrease in foraging activity (Skelly and Werner 1990, Smith et al. 2007, Schoeppner and Relyea 2008, Figiel and Semlitsch 1990). By reducing foraging activity salamander larvae should be less detectable to visual predators such as sunfish. Although our animals exposed to predator cues did not hide more, they may have moved less overall, effectively using stillness as a type of refuge. However, we did not measure activity directly.

Comparing the feeding rates in home tank water to the feeding rates in "foreign" water (from a different tank of the same treatment) allowed us to test the possibility that changing the water source caused the observed effect on feeding rate, rather than exposure to predator cues. Since there was no difference between the feeding rates in home or foreign water for animals of either treatment (Figure 4B) we can rule water change out. Finally, comparing the feeding rates in foreign water to the feeding rates in opposite water confirmed that the reduction in feeding behavior was due to predator cues. Animals raised without predator cues and then exposed to predator cues have significantly lower feeding rates than NP animals in NP water. P treatment animals with historic exposure to predators did not similarly increase their feeding rates in NP water (Figure 4C). This means that the effect of predator cues on feeding behavior, a reduction in the amount of food consumed, is immediately inducible, but not reversible in the same time frame. This suggests that exposure to predator cues has lasting effects. Decreased foraging rate

is potentially adaptive in the immediate presence of a predator, because reduced activity reduces susceptibility to detection (Sih 1992). However, our predator treatment group was chronically exposed to predator cues, and still had a reduced feeding rate. This potentially has great costs, if it leads to reduced growth (Skelly and Werner 1990). The decreased survival, smaller tail area, and smaller snout/vent length at metamorphosis that we observed may be potential costs.

Tail area was the only size metric that responded significantly to predator exposure, but in the opposite direction predicted: P treatment animals had a smaller mean tail area (Figure 4D). Past research has found that in the presence of predators, tadpoles may develop enlarged tail fins, which may act as a lure to draw predator strikes away from the more vulnerable body core (Van Buskirk et al. 2003). In some cases, tadpoles also develop conspicuous coloration or spots on the tail, which enhances its effect in drawing predator strikes (Van Buskirk et al. 2004, McCollum and Van Buskirk 1996). It is unclear whether large tail areas, with deep tail fins, also increase escape performance. Johnson et al. (2008) showed that tadpoles (*Rana sphenocephala*) with long, deep tails had the fastest burst swimming speeds, but that did not enhance survival against *Anax junius* dragonfly larvae. Van Buskirk and McCollum (2000) showed that *Anax* species dragonfly larvae induced short bodies with long, deep tails in *Hyla versicolor* tadpoles, but there was no improvement in burst swimming speed. However, odonates are sit-and-wait predators, against which fast swimming speeds might not be an effective defense. Many fish, including those in our study, are active foragers, against which fast swimming speeds might be effective in avoiding predation. Teplitsky et al. (2005) showed that stickleback fish predators induced deep tailfins and long tails in *Rana dalmatina*, and that these tadpoles have faster swimming speeds than those reared with dragonfly (*Aeshna*) larvae or no predators. Wilson et al. (2005) found that *Rana lessonae* raised with pumpkinseed sunfish (*Lepomis gibbosus*) had shallow tails with small

tail heights and higher swimming speeds than tadpoles raised in the presence of *Aeshna* larvae or without predators. The animals in our study had smaller tail areas in the presence of a *Lepomis* predator, which appears to be in agreement with the latter finding, but escape swimming performance was not measured. It is unclear whether in this case small tail area is adaptive or represents a cost of reduced feeding rates.

We expected to see plasticity of time to metamorphosis because anuran metamorphs have been shown to be more vulnerable to predation than either larvae or adults. Metamorphosis is considered to be a hazardous life stage between two different adaptive peaks (Arnold and Wassersug 1978), since metamorphs are more vulnerable to predation (Arnold and Wassersug 1978 [garter snake predators, *Thamnophis*]) due to decreased locomotor and escape performances (Wassersug and Sperry 1977, Dudley, King, and Wassersug 1991, Huey 1980). Adaptive plastic responses to predation threat at metamorphosis could manifest themselves in two ways. Predator-exposed salamanders could initiate metamorphosis earlier or they could increase the rate of development during metamorphosis, decreasing its duration. Either or both of these plastic responses could be adaptive by allowing animals faced with an aquatic predator to escape the threat of predation by completing metamorphosis earlier.

Previous theory has predicted that a perceived threat of mortality to aquatic larvae will cause amphibians to initiate developmental switches earlier and at a smaller size (Werner 1986). For example, embryos have been shown to hatch early in response to egg predators (Ireland et al. 2007, Brown-Wilusz and Landberg unpublished 2008, Capellán and Nicieza 2008). For metamorphic plasticity, however, there has been mixed support for the theory. In the red-eyed tree frog, *Agalychnis callidryas*, larval predators cause earlier metamorphosis at a smaller size, while metamorph predators cause tadpoles to metamorphose larger and later, supporting the

theoretical predictions (Vonesh and Warkentin 2006). Figiel and Semlitsch (1990) showed that even with predation by fish, spotted salamanders did not initiate metamorphosis earlier.

In studies of the effects of caged predators on the onset of metamorphosis, predator cues did not generally induce earlier metamorphosis or smaller size at metamorphosis (Relyea 2007, Benard 2004). Our findings are in agreement with this result in terms of age at metamorphic onset: animals in the P treatment group did not differ from those in the NP treatment group (Figure 5A). Again this suggests that the length of the larval period, and subsequently the age of metamorphic onset, is constrained or costly to alter (Hensley 1993). Costs of metamorphosing at small body size might include increased mortality during or after metamorphosis, or lower body condition (Walsh et al. 2008a). In addition, although non-significant, the response of the P treatment group is in the opposite direction than that predicted by theory, with predator exposed animals metamorphosing slightly later.

Animals in the P treatment group had shorter snout/vent lengths at the start of metamorphosis (Figure 5B). This may be the result of reduced growth rates due to lowered larval feeding rates in response to predator cue exposure. Although we did not see any differences in body length between predator-exposed and predator-naïve salamanders at mid-larval stages (e.g., stage 49), at stage 56 when metamorphosis begins, P treatment animals that have experienced reduced feeding rates throughout development are indeed shorter. We did not test whether this body size difference had a fitness effect during metamorphosis. Smaller metamorphs may be more vulnerable to predation because of their size, or less vulnerable if predators target larger prey. These questions have not yet been examined.

Snout/vent length and tail length were correlated with the age at metamorphic onset in opposite directions. This was a surprising result. Age at metamorphosis was positively

correlated with snout/vent length (Figure 5D), but negatively correlated with tail length (Figure 5C). This suggests that the allocation of resources to different parts of the body and different types of growth may have independent effects on the age at which animals metamorphose. Good conditions allowing an individual to grow to a large snout/vent length may favor a delay in metamorphosis to take continued advantage of a favorable environment before transitioning (Morey and Reznick 2000). In contrast, tail length may determine when individuals are capable of metamorphosis: individuals with small tail lengths may have not yet accrued enough resources to initiate metamorphosis. Thus, those individuals metamorphosing the earliest are expected to have small snout/vent lengths but long tail lengths, meaning that the larval environment has been poor for growth, but they have the capacity to begin the transition. The underlying mechanisms and timing of these opposite relationships are still unknown.

Animals never exposed to predator cues (NP), had the longest duration of metamorphosis, those exposed to predator cues throughout development (P) reduced the duration of metamorphosis, and finally, the shortest duration was for previously predator-naïve animals exposed to predator cues only during metamorphosis (NP-P; Figure 6). This means that the duration of metamorphosis, and therefore the rate of development during metamorphosis, responds plastically to predator cues. That novel predator cues during metamorphosis reduce its duration more than chronic cues may imply some degree of habituation to a constant predator threat. It is also possible that NP-P treatment animals are more physiologically competent due to their presumed better condition, so that when the need to respond to predator cues arose, they could do so better than P treatment animals. Overall, a newly perceived threat of predation reduces the duration of metamorphosis, allowing salamanders to leave the aquatic environment

earlier, minimizing the time spent in this vulnerable life stage. This is a potentially adaptive response to predator cues.

Evolutionarily, it is thought that the duration of metamorphosis should have been minimized, with little plasticity, because vulnerability to predation is so high at this stage, with both locomotor and escape performance compromised (Rose 2005, Walsh et al. 2008a). However, any mechanism that further reduces the duration of metamorphosis could be adaptive if predation rates during metamorphosis are especially high (Wassersug and Sperry 1977). The duration of metamorphosis has not been studied widely, but recent studies (limited to anurans) suggest that amphibian metamorphosis can be plastic. For instance, metamorphic duration has been shown to be influenced by temperature (Walsh et al. 2008a), snout/vent length and tail length (Downie et al. 2004), and predators (Walsh et al. 2008b). Walsh et al. (2008b) found that *Xenopus laevis* accelerated their development through metamorphosis, which agrees with our results. In contrast, Van Buskirk and Saxer (2001) found that the rate of development through metamorphosis was not affected by the presence of a predator in the water frog *Rana ridibunda*. These mixed results, and the relatively few studies that have directly examined predator-induced plasticity in the duration of metamorphosis, highlight the need for continued research on plasticity of this life stage in amphibians.

This research did not address whether the observed plasticity at metamorphosis has potential future costs. It is currently not clear why metamorphic duration is plastic if amphibians are so vulnerable to predators during this period. It is possible that there are costs associated with rapid metamorphic development, such as increased mortality or decreased locomotor performance (Walsh et al. 2008a, Arendt 1997).

Overall our results indicate that, in response to exposure to predators, spotted salamanders exhibit behavioral plasticity in feeding rates as larvae, plasticity in the size at metamorphic onset, and plasticity in the duration of metamorphosis. Although feeding behavior and refuge use have been well studied in response to predators in amphibians, metamorphosis, and the duration of metamorphosis in particular, remain understudied. Animals in the presence of an aquatic predator did not reduce larval duration, but they did reduce the duration of the vulnerable metamorphic period. Thus the spotted salamander, *Ambystoma maculatum*, can alter its rate of development during metamorphosis as a potentially adaptive response to the perceived threat of predators.

Predators are a key component of the environment that impact many aspects of development and can pose a driving force for selection. Amphibians have been shown to have the ability to distinguish many types of environmental cues, including predator cues, and alter their development in response (Rose 2005, Benard 2004). Predator presence may induce adaptive responses, but these may have costs. Understanding the intricate balance of benefit and cost to the organism, and how this dictates the specific responses observed is important to understanding how predators can affect plasticity in organisms with complex life cycles.

In the field, the pools in which salamanders develop and metamorphose may or may not have fish predators. Therefore, an understanding of the specific effects of predators on development, mortality, and metamorphosis is environmentally and evolutionarily relevant. In particular, metamorphosis, the bridge between larval and adult life stages and between the aquatic and terrestrial environment, may be very vulnerable (Arnold and Wassersug 1978). Since it also provides the opportunity for amphibians to escape the larval environment (Benard 2004), the potential for plasticity may be useful if environments shift from year to year. This

study examines the ways in which sunfish predators affect the survival, development, growth, and metamorphosis of spotted salamanders. In the future, the mechanisms underlying the changes observed and the associated costs should be explored more.

# Tables

Table 1. Continuation of the Harrison (1969) staging table for *Ambystoma maculatum* by Brown-Wilusz and Landberg (A) and Dwyer (B).

A.

| Stage | Front Limb Toe Bud | Front Limb Toe | Hind Limb Bud | Hind Limb Toe Bud | Hind Limb Toe |
|---|---|---|---|---|---|
| 44 | 0 | 2 | Yes | 0 | 0 |
| 45 | 1 | 2 | Yes | 0 | 0 |
| 46 | 0 | 3 | Yes | 0 | 0 |
| 47 | 1 | 3 | Yes | 0 | 0 |
| 48 | 1 | 3 | | 2 | 0 |
| 49 | 1 | 3 | | 1 | 2 |
| 50 | 0 | 4 | | 0 | 3 |
| 51 | 0 | 4 | | 1 | 3 |
| 52 | 0 | 4 | | 0 | 4 |
| 53 | 0 | 4 | | 1 | 4 |
| 54 | 0 | 4 | | 0 | 5 |

B.

| Stage | Tail | Gills | Coloration | |
|---|---|---|---|---|
| 54 | > 2:1 upper fin/lower fin | Full, with filaments | Light color, larval spots | Larval |
| 55 | 2:1 upper fin/lower fin | Full, with filaments | Uniformly dark | Mature Larval |
| 56 | 4:1 upper fin/lower fin | Full, with filaments | Uniformly dark, starting to mottle | Initiation of Metamorphosis |
| 57 | 10:1 upper fin/lower fin | Reduced filaments | Mottled | Peak Metamorphosis |
| 58 | 1:4 upper fin/tail no lower fin | Bare Rachis | Mottled | Peak Metamorphosis |
| 59 | 1:10 upper fin/tail | | Mottled | Continuation of Metamorphosis |
| 60 | No fin | Nub | Indistinct Spots | Continuation of Metamorphosis |
| 61 | No fin | No Nub<br><br>Gills resorbed | Spots | Adult |

Table 2. ANCOVA for development and survival

| Response | Predictors | DF | F Ratio | Prob>F |
|---|---|---|---|---|
| Tank mean stage | Clutch | 5 | 10.5040 | <0.0001 |
| | Block | 1 | 1.2562 | 0.2630 |
| (n = 429) | Treatment | 1 | 0.1649 | 0.6849 |
| | Day | 9 | 3485.664 | <0.0001 |
| Death rate | Clutch | 5 | 1.0110 | 0.4159 |
| | Block | 1 | 0.1863 | 0.6670 |
| (n = 104) | Treatment | 1 | 28.3221 | <0.0001 |
| | Day | 2 | 50.0730 | <0.0001 |
| | Treatment*Day | 2 | 3.3566 | 0.0392 |

Table 3. ANCOVA for feeding during behavior trials

| Response | Predictors | DF | F Ratio | Prob>F |
|---|---|---|---|---|
| *Home water*: | Clutch | 5 | 4.0568 | 0.0074 |
| N worms eaten | Block | 1 | 0.3831 | 0.5413 |
| | Treatment | 1 | 17.7929 | 0.0003 |
| (n = 38) | Stage | 4 | 2.9880 | 0.0373 |
| *Opposite water*, | Clutch | 5 | 0.8693 | 0.5150 |
| 90 minutes: | Block | 1 | 4.5932 | 0.0416 |
| N worms eaten | Treatment | 1 | 0.2374 | 0.6302 |
| (n = 35) | Stage | 1 | 0.3366 | 0.5668 |
| *Opposite water*, | Clutch | 5 | 0.9371 | 0.4735 |
| 1350 minutes: | Block | 1 | 0.0048 | 0.9451 |
| N worms eaten | Treatment | 1 | 0.2319 | 0.6342 |
| (n = 35) | Stage | 1 | 2.7859 | 0.1071 |
| *Home vs. foreign water*: | Clutch | 5 | 0.5132 | 0.7648 |
| | Block | 1 | 0.0367 | 0.8489 |
| N worms eaten | Treatment | 1 | 10.1732 | 0.0026 |
| | Stage | 1 | 1.2852 | 0.2629 |
| | Water type | 1 | 1.0914 | 0.3017 |
| (n = 56) | Treatment*water type | 1 | 0.4445 | 0.5084 |
| *Foreign vs. opposite water*: | Clutch | 5 | 0.9132 | 0.4811 |
| | Block | 1 | 0.0250 | 0.8750 |
| N worms eaten | Treatment | 1 | 0.2262 | 0.6367 |
| | Stage | 1 | 1.8518 | 0.1804 |
| | Water type | 1 | 9.2900 | 0.0038 |
| (n = 56) | Treatment*water type | 1 | 18.6251 | <0.0001 |

Table 4. Nominal logistic fit test of hiding during behavioral trials. Clutch, block, treatment, and stage were predictors for all tests, with water type as an additional predictor when appropriate.

| Response | DF | Chi Squared | Prob>Chi Squared |
|---|---|---|---|
| Proportion hiding: *Home water* (n = 38) | 8 | 13.81751 | 0.0866 |
| Proportion hiding: *Opposite water*, 90 minutes (n = 35) | 8 | 6.327149 | 0.6106 |
| Proportion hiding: *Opposite water*, 1350 minutes (n = 35) | 8 | 12.37478 | 0.1352 |
| Proportion hiding: *Home water vs. foreign water* (n = 56) | 9 | 10.86934 | 0.2848 |
| Proportion hiding: *Foreign water vs. opposite water* (n = 56) | 9 | 10.70425 | 0.2965 |

Table 5. ANCOVA for weight and morphometrics at the time of the feeding trials

| Response | Predictor | DF | F Ratio | Prob>F |
|---|---|---|---|---|
| Tank mean mass (n = 38) | Clutch | 5 | 2.5721 | 0.0500 |
| | Block | 1 | 0.1889 | 0.6673 |
| | Treatment | 1 | 0.5054 | 0.4833 |
| | Stage | 1 | 3.6741 | 0.0659 |
| | Tank mean snout/vent length | 1 | 8.2421 | 0.0079 |
| | Tank mean tail length | 1 | 0.0676 | 0.7968 |
| Tank mean total length (n = 38) | Clutch | 5 | 1.5269 | 0.2123 |
| | Block | 1 | 0.2968 | 0.5900 |
| | Treatment | 1 | 2.1809 | 0.1505 |
| | Tank mean stage | 1 | 23.3464 | <.0001 |
| Tank mean snout/vent length (n = 38) | Clutch | 5 | 1.3468 | 0.2730 |
| | Block | 1 | 0.3682 | 0.5487 |
| | Treatment | 1 | 2.8220 | 0.1037 |
| | Tank mean stage | 1 | 19.5009 | 0.0001 |
| Tank mean tail length (n = 38) | Clutch | 5 | 2.4681 | 0.0558 |
| | Block | 1 | 0.2195 | 0.6429 |
| | Treatment | 1 | 1.4669 | 0.2356 |
| | Tank mean stage | 1 | 20.7008 | <.0001 |
| Tank mean tail area (n = 38) | Clutch | 5 | 2.5847 | 0.0482 |
| | Block | 1 | 1.0129 | 0.3228 |
| | Treatment | 1 | 11.1340 | 0.0024 |
| | Tank mean stage | 1 | 1.3943 | 0.2476 |
| | Tank mean total length | 1 | 49.9697 | <.0001 |

Table 6. ANCOVA/ANOVA for metamorphosis data

| **Response** | **Predictor** | **DF** | **F Ratio** | **Prob>F** |
|---|---|---|---|---|
| Tank mean age | Clutch | 5 | 1.0622 | 0.3978 |
| | Block | 1 | 1.2266 | 0.2756 |
| | Treatment | 1 | 0.6990 | 0.4088 |
| | Tank mean snout/vent length | 1 | 13.3202 | 0.0008 |
| (n = 45) | Tank mean tail length | 1 | 27.3755 | <0.0001 |
| Tank mean snout/vent length | Clutch | 5 | 1.2909 | 0.2888 |
| | Block | 1 | 0.3429 | 0.5617 |
| (n = 45) | Treatment | 1 | 6.2860 | 0.0167 |
| Tank mean tail length | Clutch | 5 | 4.2727 | 0.0036 |
| | Block | 1 | 0.0149 | 0.9035 |
| (n = 45) | Treatment | 1 | 0.1831 | 0.6712 |
| Duration | Box treatment[Tank treatment] | 1 | 9.0045 | 0.0039 |
| | Clutch | 5 | 1.7621 | 0.1345 |
| (n = 125) | Block | 1 | 0.6091 | 0.4382 |

# Figures

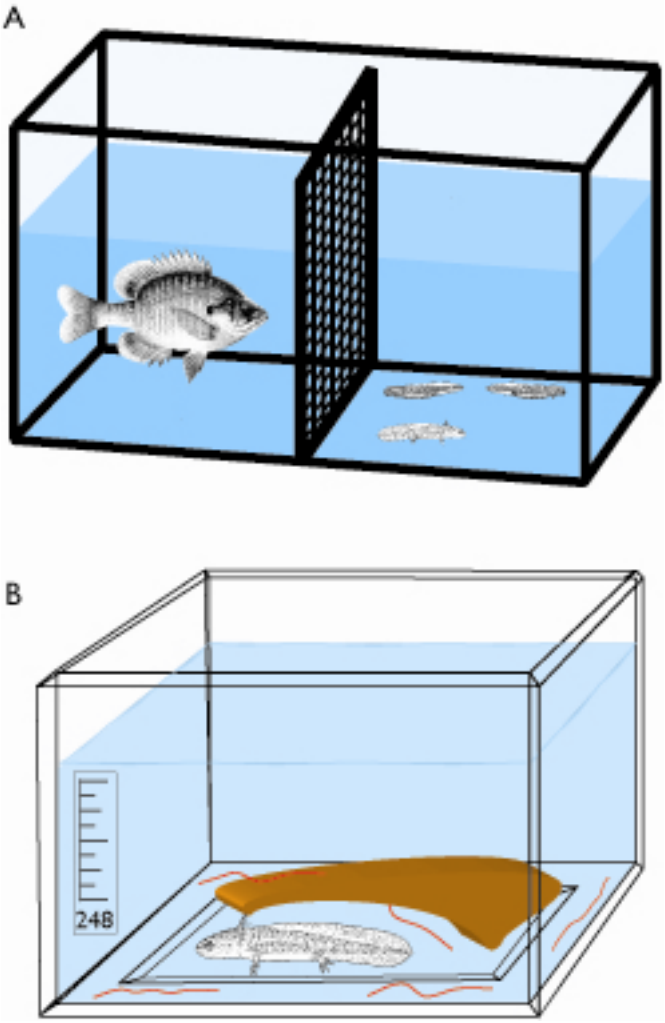


Figure 1. (A) Animals were housed in 48 38-liter (10-gallon) tanks with one of two treatments: no-predator (NP) or predator (P). Predators were separated from the experimental salamanders by a porous screen. (B) Behavioral trials took place in small acrylic boxes. Salamanders were given a concave piece of clay to hide under, and exactly five worms; hiding and feeding behavior were recorded after 90 minutes. Figures are not to scale. Drawings by Tobias Landberg.

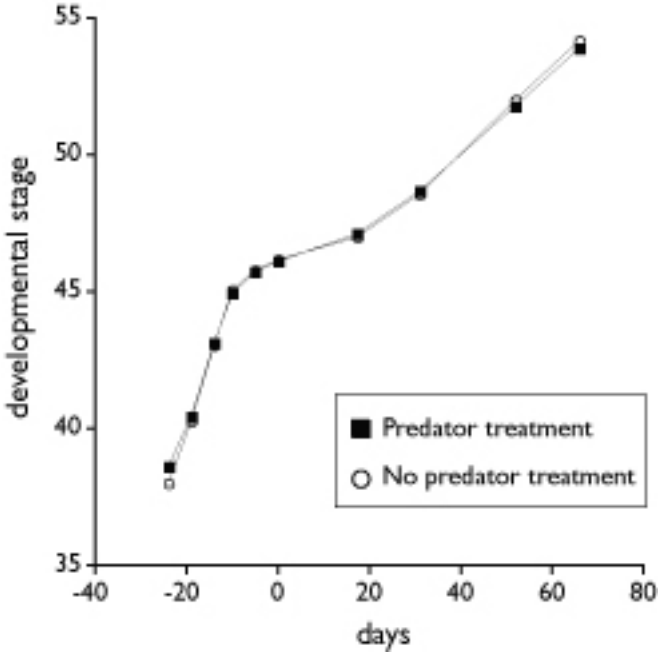


Figure 2. Tank mean stage by day represents development in the two treatment groups. There is no significant difference in the stages of animals in the no-predator (NP) and predator (P) treatment groups; as larvae, they develop at the same rate (Tukey test). Black filled squares are the data points for the P treatment, while white open circles are the data points for NP treatment. Note that day 0 is the day that all experimental salamanders were hatched.

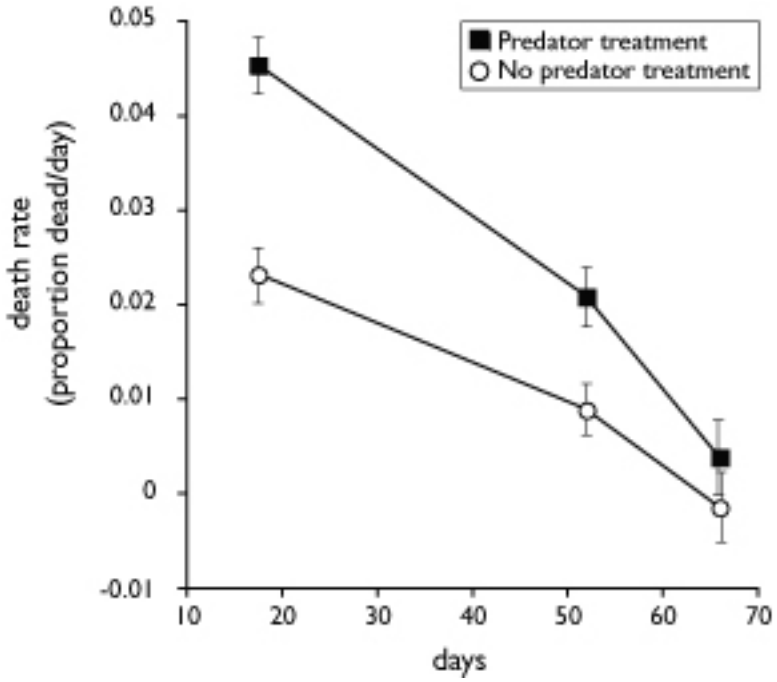


Figure 3. Death rate, the proportion dead per day, as a function of day (ANOVA adjusted tank means). The predator (P) treatment group has a higher death rate than the no-predator (NP) treatment group (Tukey test), and mortality declines over time in both treatment groups. Black filled squares are the data points for the P treatment, while white open circles are the data points for the NP treatment.

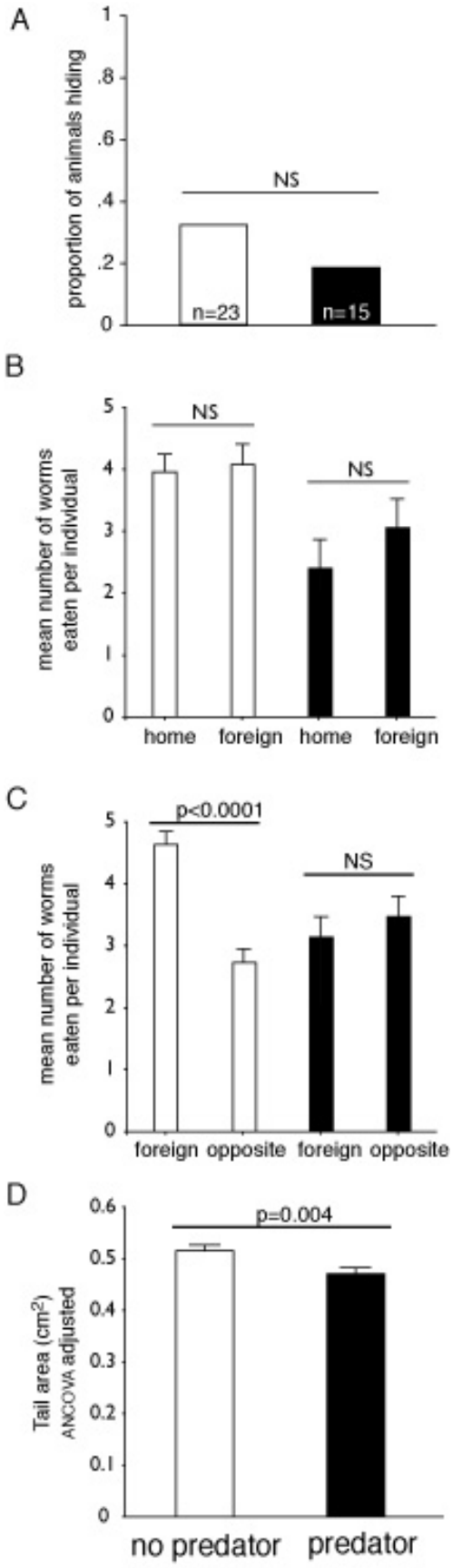


Figure 4. Behavioral Trials
White bars are the no-predator (NP) treatment, while black bars are the predator (P) treatment.

(A) The proportion of salamanders hiding at the end of 90 minutes for two treatments, in home tank water. Hiding behavior was not affected by treatment in any of the trials (nominal logistic fit tests).

(B) The mean number of worms eaten in home water vs. foreign water (same treatment) for the NP and P treatment groups. The number of worms eaten was not different for home and foreign water, across treatments (Tukey test). NP treatment animals ate significantly more than P treatment animals (Tukey test).

(C) The mean number of worms eaten in foreign water (same treatment) vs. opposite treatment water, for each treatment group. NP treatment animals significantly reduce the number of worms eaten in opposite (P treatment) water (Tukey test). For P treatment animals, there is no difference in the number of worms eaten in foreign and opposite water (Tukey test).

(D) Tail area ($cm^2$) (ANCOVA adjusted tank means) at the time of the behavioral trials for the NP and P treatments. The P treatment group has a smaller mean tail area than the NP treatment group (Tukey test).

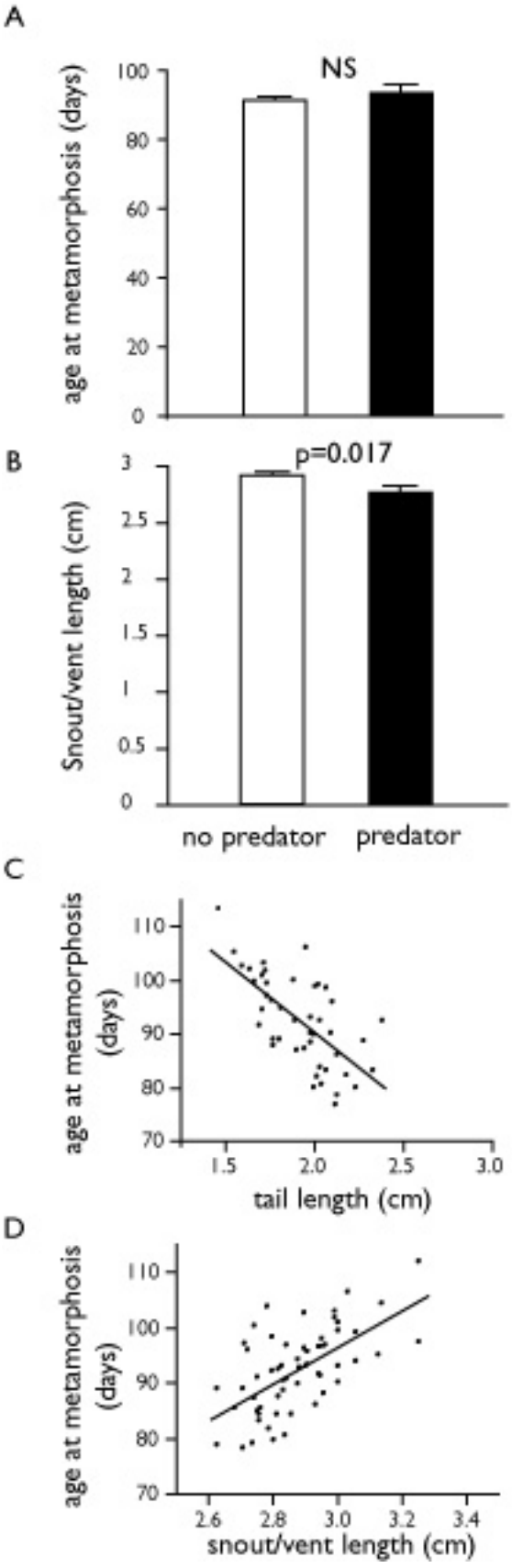


Figure 5. Onset of Metamorphosis

No-predator (NP) treatment is represented as white bars, and the predator (P) treatment is represented as black bars. Tank means are ANCOVA adjusted.

(A) The tank mean age, in days, at the onset of metamorphosis (stage 56) for the NP and P treatments did not differ (Tukey test)

(B) The tank mean snout/vent length at the onset of metamorphosis for the two treatments. The P treatment animals have shorter snout/vent lengths than the NP treatment animals when they initiate metamorphosis (Tukey test).

(C) Tank mean age at metamorphosis as a function of tail length. Across treatments, tail length inversely affects the age at metamorphosis (Tukey test; $p<0.0001$).

(D) Tank mean age at metamorphosis as a function of snout/vent length. Across treatments, snout/vent length positively affects the age at metamorphosis (Tukey test; $p=0.0008$).

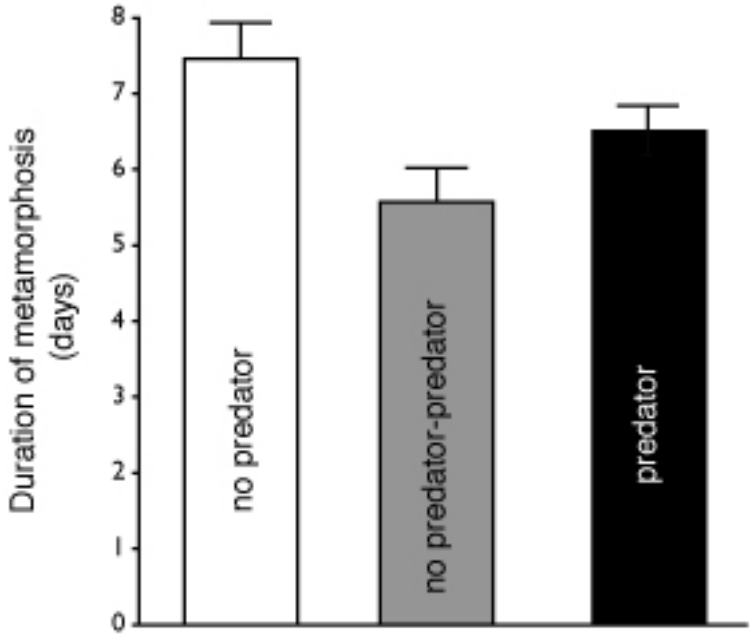


Figure 6. Duration of metamorphosis in days for each of three box treatments: no-predator (NP), no-predator-predator (NP-P), and predator (P). All three are statistically different; the duration of metamorphosis is longest in the NP treatment group, reduced in the P treatment group, and further reduced in the NP-P treatment group (Tukey test). The white bar represents the NP treatment, the grey bar the NP-P treatment, and the black bar the P treatment.

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